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Update on Fetal Transplantation: The Swedish Experience

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Summary: We implanted human embryonic mesencephalic tissue into the striatum of 13 patients with idiopathic Parkinson's disease (PD) and three patients with MPTP-induced parkinsonism. Based on our findings so far, as well as data from other groups, the following conclusions can be drawn: First, grafted dopamine (DA) neurons can survive in the human parkinsonian brain and reinnervate part of the host striatum. Second, long-term graft survival, at least up to 6 years after transplantation, is possible in PD despite a progressive degeneration of the patient's own DA neurons. Third, a majority of patients with surviving grafts show long-term improvement of therapeutic value but the symptomatic relief is, in most cases, incomplete. Presently, the most important research strategy to improve the functional recovery after transplantation is to increase the survival of grafted DA neurons and the density and extent of the dopaminergic reinnervation in the striatum. **Key Words:** Parkinson's disease—Transplantation—Dopamine neurons—Fetal tissue—Positron emission tomography.

The strategy to restore function in the damaged central nervous system by cell replacement seems particularly suitable for Parkinson's disease (PD). First, the major pathology in this disorder is a selective degeneration of the nigrostriatal dopamine (DA) system, that is, of a specific neuronal population within a restricted area of the brain. This deficit should be more easy to correct by transplantation compared with, for example, the loss of many different cell types which occurs in widespread areas of the brain in Alzheimer's disease. Second, studies in animal models of PD[1,2] have shown that grafted fetal DA neurons, taken from the ventral mesencephalon, can reinnervate the denervated striatum, restore DA transmission, and reverse some of the motor and sensorimotor deficits in parkinsonian rodents and monkeys. Since the first clinical trials with neural grafting were initiated in 1987, a total of more than 200 patients with PD worldwide have received implants of human fetal mesencephalic tissue into the striatum.[3,4] In this article, I analyze, primarily on the basis of our own findings, what has been achieved in clinical trials and which are the most important research strategies to improve the functional efficacy of neural transplantation.

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RESULTS IN CLINICAL TRIALS

On the basis of the grafting studies in patients with PD reported so far, it seems possible to draw the following conclusions:

Grafted dopamine neurons can survive and grow in the human parkinsonian brain. In almost all patients, this conclusion is based on the finding of increased striatal fluorodopa uptake after transplantation using PET. Three different research groups have reported increased fluorodopa uptake compared with preoperative scans in the grafted putamen in a total of 17 patients.[5–11] In a group of six patients with unilateral transplants,[11] we found a 68% increase of fluorodopa uptake in the grafted putamen at 8–12 months after transplantation compared with preoperatively, whereas the nongrafted putamen showed a 28% decrease (Fig. 1). Although surviving dopaminergic grafts seemed to be the most likely explanation for the increased putaminal fluorodopa uptake, this was not definitely demonstrated until the recent histopathologic studies by Kordower and coworkers.[12,13] They showed, in a parkinsonian patient who died 18 months after transplantation surgery, that increased fluorodopa uptake on PET was associated with graft survival and reinnervation of the host striatum, and that no sprouting had occurred from the patient's own DA neurons.

In contrast to putaminal implants, no clear-cut PET evidence for survival of dopaminergic grafts in the caudate has so far been obtained[9,11] (Fig. 1). The reason is

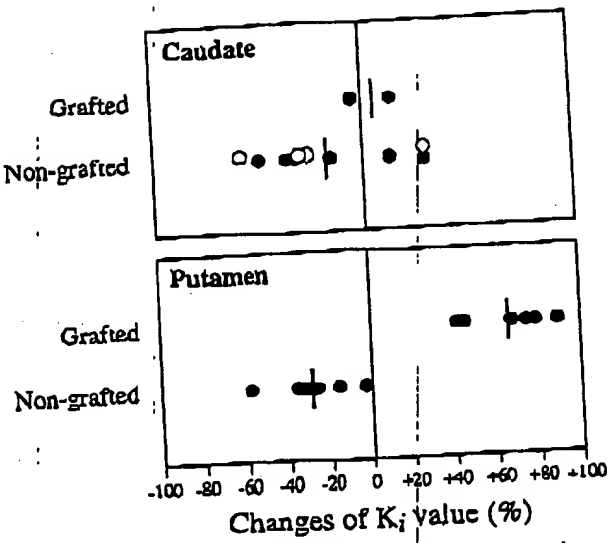

FIG. 1. Survival of unilateral intrastriatal grafts of fetal ventral mesencephalic tissue as assessed using PET. Tissue from 4–7 donors was implanted either in the putamen alone (4 cases) or in the caudate + putamen (2 cases) in 6 patients with idiopathic PD. This summary diagram shows the percent change of fluorodopa uptake (K_i value) compared with preoperatively in the grafted and nongrafted striatal regions at 8–12 months after transplantation. Both data from individual cases and mean changes for all patients are given. Values for caudate nucleus depicted as open circles are from the side ipsilateral to the grafted putamen in the four patients in whom the caudate nucleus was not grafted. Data from Wenning GK, Odin P, Morrish P, et al. Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. *Ann Neurol.* 1997;42:95–107.

unclear, but it can not be excluded that the conditions for graft survival are more favorable in the putamen, for example, resulting from better vascularization.

Folkerth and Durso[14] recently described autopsy findings in a patient with PD who had received intraventricular infusion of "diencephalic/mesencephalic region cells" from an early gestational (5–6 week) human fetus. At 23 months after grafting, there were no surviving DA neurons but marked overgrowth of nonneuronal tissues was found. This adverse effect, which has not been observed in any other patient, can be avoided by implanting only the specific part of the fetal ventral mesencephalon containing the dopaminergic cell bodies and by meticulously removing the meninges. Both animal studies and clinical trials show that with these precautions, the risk for uncontrolled growth of graft tissue is negligible.

Reproducible dopamine graft survival can be obtained with currently used transplantation procedures. In our series of patients with intrastriatal fetal mesencephalic implants, repeated PET scans have shown increased fluorodopa uptake in the grafted putamen in all examined cases except the first two patients (that is, in a total of 12 patients).[5–8,11,15] Similarly, Freeman et al.[10] and Remy et al.[9] reported increased fluorodopa uptake in 4 of 4 and 5 of 5 grafted patients, respectively. It seems likely that PET demonstrates surviving dopaminergic grafts in these patients.

Long-term graft survival, at least up to 6 years, is possible in progressive Parkinson's disease. Idiopathic PD is an ongoing degenerative disorder and it therefore seems possible that the fetal neural grafts also could be destroyed by the disease process. In two patients of our own series, who were transplanted unilaterally in the left and right putamen, respectively, the fluorodopa uptake in the grafted putamen was still high 6 years after surgery (Fig. 2B).[6,11] However, there was also a fall of tracer uptake in nongrafted striatal regions indicating degeneration of the patient's own DA neurons.

Long-term immunosuppression is not necessary for graft survival. In nine patients from three different research groups, withdrawal of cyclosporin at 6–31 months after transplantation did not interfere with graft survival as assessed 6 months to 3 years later using PET or histopathology.[6,11,12,16] However, it is not yet known if short-term immunosuppression is required for graft survival. In fact, all patients who have shown graft survival on PET have received immunosuppression at the time of transplantation and for at least 6 months thereafter.

Grafts can give rise to long-term symptomatic relief of therapeutic value to patients with Parkinson's disease, but the symptomatic relief is in most patients incomplete with respect to both the degree and pattern of functional recovery. Therapeutic improvement associated with graft survival, as observed with PET and in one case also with histopathology, has been reported by three different research groups in Paris, France; Tampa, Florida, U.S.A.; and Lund, Sweden.[5–13,15–17] In our six unilaterally grafted patients, we observed improvements of clear therapeutic value in four cases, whereas the changes in two patients were modest[11] (Fig. 3). The evolution and pattern of functional recovery exhibited certain common features. After a delay of 2–3 months, often with an initial worsening of parkinsonian symptoms, the patients started to improve. There was increased percent time in the "on" phase. Tremor did not improve but rigidity and hypokinesia decreased during "off" phases, bilaterally, predominantly in the arm contralateral to the graft. The quality of movement also improved contralaterally. Gait seemed to improve in patients with both caudate and putamen implants but not in cases with putamen grafts only. Even if some positive effects were found, more data are needed to clarify whether fetal dopaminergic grafts can improve dyskinesias and postural dysfunction in parkinsonian patients.

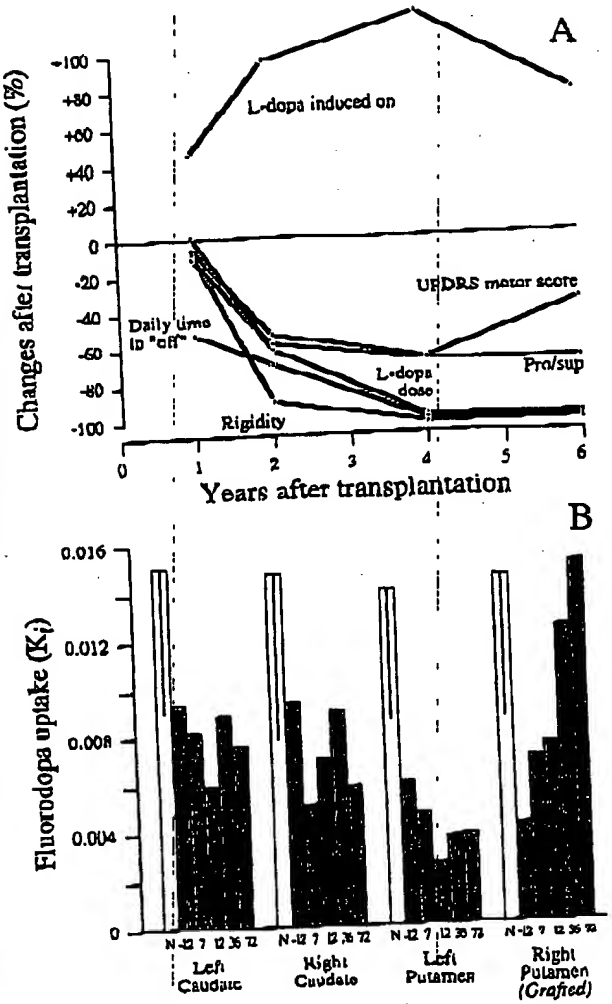

FIG. 2. Long-term survival and function of unilateral dopaminergic grafts as illustrated in a patient operated in the right putamen. (A) The patient improved after transplantation with increased duration of L-dopa-induced "on" phase, reduced UPDRS motor score and time in the "off" phase, and decreased rigidity and more rapid performance of timed motor tasks (pronation/supination) in "off." L-dopa was withdrawn after 32 months. However, during the sixth postoperative year, there was a moderate worsening of parkinsonian symptoms. (B) Before grafting, fluorodopa uptake was pathologic bilaterally in the putamen. After transplantation, there was a progressive increase of fluorodopa uptake in the grafted putamen, and uptake was normalized at 6 years. Comparative data are given for a group of 17 healthy volunteers (N; error bars represent normal mean -2 SD). Numbers on X-axis refer to months before (minus) and after surgery. Data from Wenning GK, Odin P, Morrish P, et al. Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. *Ann Neurol.* 1997;42:95–107.

The long-term functional effects of neural transplantation are illustrated by the findings in one of our patients who was grafted in the right putamen.[6,11] He improved up to 2 years after transplantation (Fig. 2A). "On–off" fluctuations and hypokinesia and rigidity on the side contralateral to the graft almost completely disappeared. L-dopa could be withdrawn at 32 months after surgery. In complete agreement with the marked clinical improvement, he showed a normalization of fluorodopa uptake in the grafted putamen at 6 years (Fig. 2B). However, during the sixth postoperative year, there was a moderate worsening of parkinsonian symptoms, even if the patient was still significantly improved compared with his con-

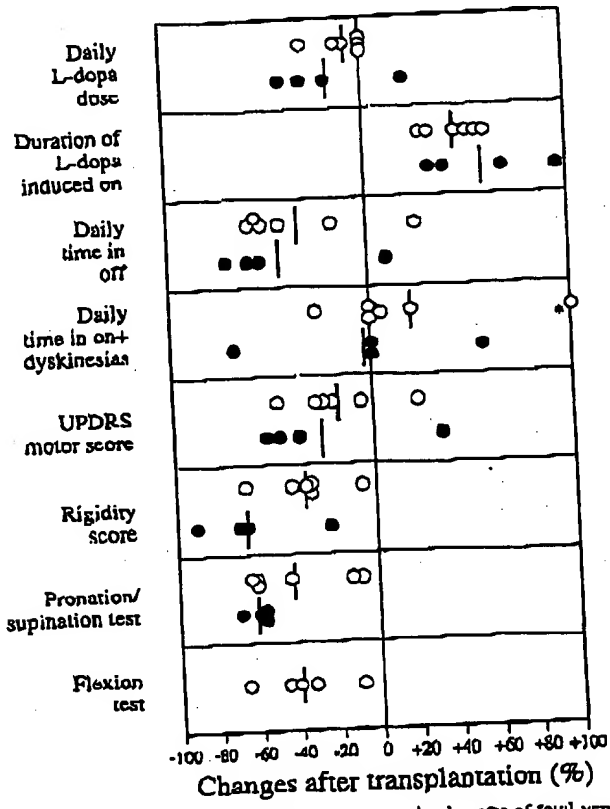

FIG. 3. Functional effects of unilateral intrastriatal grafts of fetal ventral mesencephalic tissue (same patients as in Fig. 1). Summary diagram showing the percent change during the first (open circles; 6 cases) and second (filled circles; 4 cases) postoperative year of daily L-dopa dose (at the end of each year), and of the results from autoscoring and various neurologic and neurophysiological assessments (mean or median values) in the "off" phase. Values for individual patients (circles) and means for the entire group (bars) are given for each parameter. Rigidity, pronation/supination, and flexion test data are from the arm contralateral to the graft. * = +128%. Data from Wenning GK, Odin P, Morrish P, et al. Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. *Ann Neurol.* 1997;42:95–107.

dition preoperatively (Fig. 2A). Probably the disease process had led to a further degeneration of the patient's remaining DA neurons and perhaps also other brain pathology, which had counteracted the continuing improvement induced by the unilateral graft.

Further evidence for a graft-induced effect on the host brain, mediated through restoration of dopaminergic function in the denervated striatum, has been obtained using $H_2{}^{15}O$-PET activation scans. In one of our patients, who showed marked clinical improvement and increased fluorodopa uptake in the grafted putamen, we found a 2.8-fold increase of the movement-related change of rCBF in the rostral supplementary motor area (SMA) (Ceballos-Baumann et al., to be published). Similar effects have previously been observed after administration of the DA agonist apomorphine to patients with PD.[10] Our findings suggest that the dopaminergic grafts had led to a functional reafferentation of the rostral SMA.

STRATEGIES TO DEVELOP TRANSPLANTATION THERAPY

The demonstration of survival and function of grafted dopaminergic neurons represents an important step toward transplantation therapy in PD. However, it must be underscored that the symptomatic relief needs to be improved and we must become independent of a continuous supply of large amounts of human fetal tissue before neural grafting will become an established treatment in PD. Two of the most important research strategies to pursue in animal studies and clinical trials will be discussed in the following paragraphs.

To increase dopamine neuron survival and reinnervation in the striatum. The survival of fetal DA neurons is low, only 5–20% after grafting with available procedures. Therefore, mesencephalic tissue from 3–4 fetuses needs to be implanted per side to induce a major improvement in a patient,[3] which severely restricts the clinical usefulness of neural grafting. In our ongoing patient series, we are supplying the graft with the lazaroid, tirilazad, which inhibits free radical processes. In vitro data on both rat and human DA neurons as well as in vivo data with rat mesencephalic grafts show two- to threefold increase of DA neuron survival with lazaroids.[19,20]

In the clinical studies performed thus far, only part of the denervated striatum (mainly putamen, often unilaterally) has been reinnervated, which may to some extent explain the incomplete functional recovery. Animal experimental data, as well as the histopathologic analysis in a parkinsonian patient,[12,13] show that the reinnervation provided by a dopaminergic graft is restricted and, from each human implant, extends approximately 5–7 mm from the graft into the host striatum. Kordower et al.[13] estimated that the grafted dopaminergic neurons had reinnervated approximately 30–50% of the postcommissural putamen in their patient. Thus, the reinnervation is incomplete with present transplantation procedures. To increase the volume of reinnervation in the putamen and caudate nucleus, and to reach other areas also (for example, the ventral striatum), which most likely would lead to improved symptomatic relief, the number of implants has to be increased or the axonal outgrowth must be stimulated.

A future strategy to increase DA neuron survival and axonal outgrowth in patients might be to administer a trophic factor, for example, brain-derived neurotrophic factor (BDNF) or glial cell line-derived neurotrophic factor (GDNF). This idea is supported by recent animal experimental data. Yurek and coworkers[21] implanted rat fetal mesencephalic tissue into the DA-denervated rat striatum and then infused BDNF close to the graft for 4 weeks. Six weeks later, the area of dopaminergic fiber outgrowth from the graft was significantly larger in the BDNF-treated compared with the control-infused groups. In a similar experiment, Rosenblad et al.[22] gave intrastriatal injections of GDNF every third day during the first 3 weeks after grafting. The GDNF-treated rats showed a more rapid functional recovery, and at 4 weeks, both the number of surviving DA neurons and the density of their fiber outgrowth were markedly higher compared with vehicle-injected control subjects.

To use xenografts of fetal dopamine neurons. The problem with the need of large amounts of fetal mesencephalic tissue might possibly be solved by the use of xenografts, for example, from pigs. It has been demonstrated that intrastriatal dopaminergic grafts of fetal porcine ventral mesencephalon can survive, reinnervate the denervated striatum, and reverse drug-induced rotational asymmetry in hemiparkinsonian rats.[23] Recently, Deacon et al.[24] reported survival of grafted pig dopaminergic neurons in an immunosuppressed patient with PD who died 7½ months after transplantation. A total of 638 dopaminergic neurons were found at three graft sites in the putamen. This finding supports the idea that xenografts might be useful in the clinical setting. However, both the yield of surviving pig dopaminergic neurons and their volume of reinnervation in the host striatum have to be markedly increased to make the xenograft approach comparable to that using human fetal neurons.

CONCLUSIONS

Clinical trials with neural transplantation have provided good evidence that fetal dopaminergic grafts can, at least partly, restore striatal DA transmission in patients

with PD and give rise to therapeutically valuable symptomatic relief. Most importantly, these findings indicate that the basic principle to restore function by cell replacement works also in the 50- to 60-year-old diseased human brain and in PD for at least 6 years. However, neural grafting is still an experimental procedure. Research in this field should aim, first, at increasing the functional efficacy of the grafts and, second, at reducing the need for large amounts of human fetal tissue, possibly by finding alternative sources of cells useful for transplantation.

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REFERENCES

1. Brundin P, Duan W-M, Sauer H. Functional effects of mesencephalic dopamine neurons and adrenal chromaffin cells grafted to the rodent striatum. In: Dunnett SB, Björklund A, eds. *Functional neural transplantation*. New York, NY: Raven Press, 1994:9–40.
2. Annett LE. Functional studies of neural grafts in parkinsonian primates. In: Dunnett SB, Björklund A, eds. *Functional neural transplantation*. New York, NY: Raven Press, 1994:71–102.
3. Lindvall O. Neural transplantation in Parkinson's disease. In: Dunnett SB, Björklund A, eds. *Functional neural transplantation*. New York, NY: Raven Press, 1994:103–137.
4. Olanow CW, Kordower JH, Freeman TB. Fetal nigral transplantation as a therapy for Parkinson's disease. *TINS* 1996;19:102–109.
5. Lindvall O, Brundin P, Widner H, et al. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. *Science* 1990;247:574–577.
6. Lindvall O, Sawle G, Widner H, et al. Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. *Ann Neurol* 1994;35:172–180.
7. Sawle G, Bloomfield PM, Björklund A, et al. Transplantation of fetal dopamine neurons in Parkinson's disease: positron emission tomography [^{18}F]-6-L-fluorodopa studies in two patients with putaminal implants. *Ann Neurol* 1992;31:166–173.
8. Widner H, Tetrud J, Rehncrona S, et al. Bilateral fetal mesencephalic grafting in two patients with parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *N Engl J Med* 1992;327:1556–1563.
9. Remy P, Samson Y, Hantraye P, et al. Clinical correlates of [^{18}F] fluorodopa uptake in five grafted parkinsonian patients. *Ann Neurol* 1995;38:580–588.
10. Freeman TB, Olanow CW, Hauser RA, et al. Bilateral fetal nigral transplantation into the postcommissural putamen in Parkinson's disease. *Ann Neurol* 1995;38:379–388.
11. Wenning GK, Odin P, Morrish P, et al. Short- and long-term survival and function of unilateral intrastriatal dopaminergic grafts in Parkinson's disease. *Ann Neurol* 1997;42:95–107.
12. Kordower JH, Freeman TB, Snow BJ, et al. Neuropathological evidence of graft survival and striatal reinnervation after the transplantation of fetal mesencephalic tissue in a patient with Parkinson's disease. *N Engl J Med* 1995;332:1118–1124.
13. Kordower JH, Rosenstein JM, Collier TJ, et al. Functional fetal nigral grafts in a patient with Parkinson's disease: chemoanatomic, ultrastructural and metabolic studies. *J Comp Neurol* 1996;370:203–230.
14. Folkerth RD, Durso R. Survival and proliferation of nonneural tissues, with obstruction of cerebral ventricles, in a parkinsonian patient treated with fetal allografts. *Neurology* 1996;46:1219–1225.
15. Lindvall O, Widner H, Rehncrona S, et al. Transplantation of fetal dopamine neurons in Parkinson's disease: one-year clinical and neurophysiological observations in two patients with putaminal implants. *Ann Neurol* 1992;31:155–165.
16. Peschanski M, Defer G, N'Guyen JP, et al. Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of fetal ventral mesencephalon. *Brain* 1994;117:487–499.
17. Defer G-L, Geny C, Ricolfi F, et al. Long-term outcome of unilaterally transplanted parkinsonian patients. I: Clinical approach. *Brain* 1996;119:41–50.
18. Brooks DJ, Jenkins IH, Passingham RE. Positron emission tomography studies on regional cerebral control of voluntary movement. In: Mano N, Hamada I, Delong MR, eds. *Role of the cerebellum and basal ganglia in voluntary movement*. New York, NY: Excerpta Medica, 1993:267–274.
19. Nakao N, Frodl EM, Duan W-M, Widner H, Brundin P, Lazarovids improve the survival of grafted rat embryonic dopamine neurons. *Proc Natl Acad Sci USA* 1994;91:12408–12412.
20. Ohlberg A, Keep M, Brundin P, Lindvall O. Tirilazad mesylate improves survival of rat and human embryonic mesencephalic neurons in vitro. *Exp Neurol* 1997;147:498–502.
21. Yurek D, Lu W, Hipkens S, Wiegand SJ. BDNF enhances the functional reinnervation of the striatum by grafted fetal dopamine neurons. *Exp Neurol* 1996;137:105–118.
22. Rosenblad C, Martinez-Serrano A, Björklund A. Glial cell line-derived neurotrophic factor increases survival, growth and function of intrastriatal fetal nigral dopaminergic grafts. *Neuroscience* 1996;75:979–985.
23. Galpern WG, Burns LH, Deacon TW, Dinsmore J, Isacson O. Xenotransplantation of porcine fetal ventral mesencephalon in a rat model of Parkinson's disease: functional recovery and graft morphology. *Exp Neurol* 1996;140:1–13.
24. Deacon T, Schumacher J, Dinsmore J, et al. Histological evidence of fetal pig neural cell survival after transplantation into a patient with Parkinson's disease. *Nat Med* 1997;3:350–353.